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Abstract: OBJECTIVE: To evaluate the most common bacterial pathogens associated with septic keratitis in veterinary patients from Switzerland. The second objective was to analyze antibiotic susceptibility test results of the identified bacterial pathogens. The third objective was to evaluate potential breed predispositions to septic keratitis. PROCEDURES: Two hundred and fifty-five cultures and antibiotic susceptibility reports from dogs, cats, and horses with septic keratitis that were presented to the University of Zurich Veterinary Medical Teaching Hospital between 2009 and 2013 were reviewed. Odds ratios for the risk of having a septic keratitis were estimated for all dog and cat breeds compared to the general nonbrachycephalic hospital population. RESULTS: Ninety-six, 29, and 31 positive cultures were obtained from 89 canine, 28 feline, and 29 equine eyes, respectively. Repeat sampling accounted for the differences in numbers. Negative culture results were obtained in 50, 31, and 18 cases. Staphylococci and streptococci accounted for 66% of the isolates in dogs and 80% of the isolates in cats and horses. Staphylococcus spp. had a higher percentage of fluoroquinolone-resistant isolates compared to previous reports. Brachycephalic breeds had elevated odds ratios for the presence of septic keratitis. CONCLUSION: Identified bacterial pathogens and their prevalence as well as the elevated odds ratios for septic keratitis in brachycephalics are roughly consistent with previous studies. Based on systemic breakpoint data, resistance to commonly used topical antibiotics, including the second-generation fluoroquinolones, was found.

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Septic keratitis in dogs, cats, and horses in Switzerland: associated bacteria and antibiotic susceptibility

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Abstract

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Procedures Two hundred and fifty-five cultures and antibiotic susceptibility reports from dogs, cats, and horses with septic keratitis that were presented to the University of Zurich Veterinary Medical Teaching Hospital between 2009 and 2013 were reviewed. Odds ratios for the risk of having a septic keratitis were estimated for all dog and cat breeds compared to the general nonbrachycephalic hospital population.

Results Ninety-six, 29, and 31 positive cultures were obtained from 89 canine, 28 feline, and 29 equine eyes, respectively. Repeat sampling accounted for the differences in numbers. Negative culture results were obtained in 50, 31, and 18 cases. Staphylococci and streptococci accounted for 66% of the isolates in dogs and 80% of the isolates in cats and horses. *Staphylococcus spp.* had a higher percentage of fluoroquinolone-resistant isolates compared to previous reports. Brachycephalic breeds had elevated odds ratios for the presence of septic keratitis.

Conclusion Identified bacterial pathogens and their prevalence as well as the elevated odds ratios for septic keratitis in brachycephalics are roughly consistent with previous studies. Based on systemic breakpoint data, resistance to commonly used topical antibiotics, including the second-generation fluoroquinolones, was found.

Key Words: bacterial resistance, cat, dog, horse, susceptibility test, ulcerative keratitis

INTRODUCTION

Corneal infections in the form of malacic ulcers are common and potentially devastating diseases treated by veterinary- and human ophthalmologists alike.[1–3] Many literature references support this statement, but precise prevalence numbers have not been published in the veterinary literature.[4–8] Almost 20% (2194/12085) of all ophthalmic small animal patients and 30% (548/1858) of all ophthalmic equine patients seen in our clinic during the last 20 years presented with a corneal defect. (Patient database 1995–2014, Section of Veterinary Ophthalmology, Vetsuisse Faculty, University of Zurich).

Bacterial infection and the subsequent inflammation of the cornea can cause keratomalacia/corneal melting, a result of activated enzymes such as collagenases, elastases, matrix metalloproteinases (MMPs), and cathepsins and an imbalance between these proteolytic enzymes and the proteinase inhibitors present in the cornea and precorneal tear film.[9,10] The malacic process can lead to corneal ulcer deepening, descemetocele formation, and corneal perforation.[4,11]

Previous studies demonstrated that the most frequently isolated species of bacteria causing corneal infections are *Staphylococcus spp.*, *Streptococcus spp.*, and *Pseudomonas spp.* in dogs;[8,12–14] and *Pseudomonas spp.*, *Streptococcus spp.*, and *Staphylococcus spp.* in horses.[15–17] The most frequently isolated species of bacteria from the ocular surface of healthy cats were *Staphylococcus spp.*, *Pseudomonas spp.*, *Pasteurella spp.*, and beta-hemolytic *Streptococcus spp.*[18]

A first-choice antibiotic for treatment of ocular surface infections in our clinic is a triple-antibiotic formulation containing neomycin, polymyxin B, and bacitracin or gramicidin. Other topically applied antibiotics used for

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corneal diseases in veterinary ophthalmology include tobramycin, gentamicin, ciprofloxacin, ofloxacin, moxifloxacin, chloramphenicol, oxytetracycline, and erythromycin.[19]

In a 2006 study of 97 dogs with bacterial keratitis, Tolar *et al.*[8] observed no significant changes in the resistance patterns to selected antibiotics over an 11-year period from 1993 to 2003. Nonetheless, a number of studies of bacterial keratitis report antibacterial resistance development in veterinary medicine,[15,17] and in human medicine.[20–23]

The primary objective of this study was to evaluate the most common bacterial pathogens associated with septic keratitis in veterinary patients from Switzerland. The second objective was to analyze the susceptibility of the identified bacterial pathogens to antibiotics, including a potential shift in prevalence and antibiotic resistance over time. The third objective was to evaluate potential breed predispositions to septic keratitis within the hospital population. Patients originated from all sections of the country and from neighboring countries, offering a good regional overview.

MATERIALS AND METHODS

Case selection: inclusion and exclusion criteria

The reports of 425 samples obtained from dogs, cats, and horses submitted for bacteriologic culture and antibiotic susceptibility testing by the ophthalmology section at the Vetsuisse Faculty, University of Zurich, between January 2009 and December 2013 were collected. Reports were included if the case history (OblonData Praxissoftware) identified a corneal defect including keratitis, erosion, simple-, complicated- or malacic ulcer, descemetocele, corneal laceration or perforation, corneal abscess, or corneal foreign body; and the case history also suggested a clinical suspicion for a septic process. Reports were excluded if a case history was not available or yielded inadequate information or if an antibiotic susceptibility test was not available (22 reports), if the patient was not diagnosed with a keratopathy (138 reports), or in cases that were culture positive for *Mycoplasma spp.*, *Chlamydophila spp.*, or *Leptospira spp*. (10 reports).

Sampling and sample processing

Samples were collected from corneas with sterile swabs, after administration of one drop of topical anesthetic. Oxybuprocaine (Novesin 0.4%, OmniVision) was used in dogs and cats, and proxymetacaine (Alcaine, Alcon) was used in horses. Swabs were kept in Amies Medium after sample collection until further analysis. Most samples were processed by IDEXX Diavet AG (Bäch, Switzerland). In some cases, samples were processed by the Institute of Veterinary Bacteriology (IVB), University of Zurich, and one case was analyzed by the Institute of Veterinary Bacteriology (ZOBA), University of Berne. Samples were inoculated on- and into various culture media: Columbia Agar with 5% sheep blood, MacConkey Agar, Columbia CNA agar with 5% sheep blood, Schaedler Agar with 5% sheep blood, Schaedler Bouillon for enrichment/accumulation (culture media by BD: Becton Dickinson and Company). After a 24- to 72-h incubation period, individual colonies were further analyzed using standard bacteriological procedures (gram stain, catalase test, oxidase test, PYR test, oxidation–fermentation test, agglutination test for coagulase activity, agglutination test for the classification of *Streptococcus spp*. Lancefield groups and growth performance on various agars). Additionally, colonies were identified using the Vitek® (Biomérieux, Marcy l'Etoile, France) automated microbial identification system.

Antibiotic susceptibility testing was performed using the agar disk diffusion test (Mueller Hinton Agar with 5% sheep blood by BD) or by Vitek®. The results were interpreted in accordance with the 2009 CLSI (clinical laboratory standards institute) standards. When no inhibition zone standards were available for an antimicrobial agent, manufacturer information was used as reference. It should be noted that CLSI and manufacturer breakpoints used to define resistance for topically applied antibiotics are not available and may differ considerably from systemic breakpoints.[24,25]

Isolates were identified as methicillin-resistant *Staphylococcus aureus* (MRSA) or methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) via positive growth on specialized culture medium (Brilliance MRSA 2 Agar) and PBP2a (penicillin binding protein 2A) expression.

Only samples where bacteria were identified to the species level, or those with proven hemolysis were included in the final antibiotic susceptibility results' evaluation.

Antibiotics selected for susceptibility testing

The following antibiotics (or antibiotic classes) were included in the analysis of antibiotic susceptibility, due to their use in topical treatment protocols for septic keratitis in veterinary ophthalmology: cephalosporin (depending on the year of testing cefoperazone, cefalexin, cefoxitin, or cefovecin was included), neomycin, gentamicin, tobramycin, tetracycline, colistin (as substitute for polymyxin B), enrofloxacin, norfloxacin, marbofloxacin (due to their partial cross-resistance with ofloxacin, ciprofloxacin, and moxifloxacin), fusidic acid, and bacitracin. Antibiotics for which a variable effectivity against certain bacterial groups has classically been observed were marked '*: variable susceptibility' in the figures. Antibiotics against which, due to pharmacological mechanisms, resistance occurs naturally within certain bacterial groups were marked '#: intrinsic resistance' in the figures.

Statistics

Because this study is descriptive in nature, limited statistical analyses were performed. Odds ratios (ORs) and associated 95% confidence intervals (CIs) were estimated

for all dog and cat breeds included in the study population. The ORs thus represent the chance for a dog of a specific breed to be presented with a septic corneal disease (as specified by the criteria in the Materials and Methods section) in comparison with dogs of non-brachycephalic breeds within the general hospital population. Boxers, English Bulldogs, French Bulldogs, King Charles Spaniels, Pekingeses, Boston Terriers, Continental Bulldogs, Shih Tzus, and Pugs were considered to be brachycephalic dog breeds.[26–28] Persian cats were compared with non-Persian (mostly European shorthaired) cats, as the Persian cat was the only brachycephalic cat breed analyzed.[29] Additionally, for description of the breed-specific prevalences, binomial 95% confidence intervals were estimated. Here, the estimated breed-specific prevalences indicate the proportion of affected individuals within the hospital population. The statistics program R 3.1.2. was used for all statistical calculations (R Core Team (2016). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/).

RESULTS

Two hundred and fifty-five culture and antibiotic susceptibility reports were reviewed. Ninety-six, 29, and 31 positive cultures were obtained from 89 canine, 28 feline, and 29 equine eyes, respectively. Repeated sampling of patients (contralateral eye or ipsilateral eye but different septic keratitis episode) accounted for the differences in number of cultures and eyes. Negative cultures were obtained from 34% (50/146) canine, 52% (31/60) feline, and 37% (18/49) equine samples.

Bacterial isolates

The isolates were not specified to exact bacterial species in every case, resulting in a high number of isolates that were specified up to genus level (e.g., *Staphylococcus* spp.) or further specified only to a limited degree (e.g., hemolytic *Staphylococcus* spp.).

In the staphylococci group, hemolytic *Staphylococcus spp*. would likely be *Staphylococcus (S.) aureus*, *S. pseudintermedius*, or *S. hemolyticus*. According to recent systematic research, *S. intermedius* is referred to as *S. pseudintermedius*.[30,31] Consequently, all results mentioning *S. intermedius* were interpreted as *S. pseudintermedius*.

The most common bacterial isolates in canine patients included *Staphylococcus spp*. (46 of 113 total isolates [40%]), *Streptococcus spp*. (29/113 [25%]), *Pseudomonas spp*. (12/113 [10%]), *Coliform spp*. (9/113 [8%]), and *Pasteurella spp*. (8/113 [7%]) (see Table 1). The most commonly isolated *Staphylococcus sp*. was *S. pseudintermedius* (14/46 *Staphylococcus spp*.). One of the *S. pseudintermedius* isolates was highly suspicious to be classified as methicillin-resistant *Staphylococcus pseudintermedius* (MRSP), and three isolates were identified as MRSP. Beta-hemolytic streptococci represented 76% (22/29) of all *Streptococcus* isolates.

The most common bacterial infections in feline patients included *Staphylococcus spp*. (21/32 [65%]), *Streptococcus spp*. (4/32 [12%]) and *Pasteurella spp*. (2/32 [6%]), or aerobic spore-forming bacteria (2/32 [6%]) (see Table 1). Two of the feline *Staphylococcus spp*. isolates were suspicious for methicillin resistance. One of the S. *pseudintermedius* isolates was confirmed as MRSP.

No difference in prevalence of bacterial genera was observed between brachycephalic breeds and mesocephalic breeds in either dogs or cats (data not shown).

The most common bacterial isolates in equine patients included *Streptococcus spp*. (16/35 [45%]), *Staphylococcus spp*. (12/35 [34%]), and *Pseudomonas spp*. (2/35 [5%]) or Coliform bacteria (2/35 [5%]) (see Table 1). One of the equine Staphylococcus isolates was suspicious for methicillin resistance. One of the *S. pseudintermedius isolates* was confirmed as MRSP.

Antibiotic susceptibility

There is a difference in the number of isolates and number of susceptibility test results because susceptibility testing was not performed on each isolate. This typically applied to the individual bacterial species identified in mixed infections, where only the suspected pathogen(s) were tested. (Figs 1–3).

Using systemic breakpoint data, resistance to enrofloxacin/norfloxacin was observed in 48% (15/31) of all staphylococci and 19% (4/21) of all streptococci isolated from dogs, with an additional 9% (3/31) and 52% (11/21) demonstrating intermediate susceptibility, respectively.

Resistance to gentamicin was observed in 23% (dogs 7/31), 7% (cats 1/14), and 50% (horses 2/4) of all staphylococci and in 95% (dogs 20/21) to 100% (horses 9/9, cats 3/3) of all *Streptococcus spp*. No *P. aeruginosa* isolates from dogs were found to be resistant to gentamicin. A similar resistance profile was observed for neomycin: 23% (dogs 7/31), 14% (cats 2/14), and 50% (horses 2/4) of all staphylococci, all streptococci and 16% (2/12) of *P. aeruginosa* isolates from dogs were neomycin resistant.

Resistance to tetracycline was observed in 48% (dogs 15/31), 14% (cats 2/14), and 75% (horses 3/4) of all *Staphylococcus spp*.; and 68% (dogs 15/22) and 44% (horses 4/9) *of all Streptococcus spp*.

Resistance to cephalosporins was observed in 16% (5/31) of all *Staphylococcus spp*. from dogs (14% cats, 2/14; 75% horses, 3/4). All *Streptococcus spp*. were susceptible. Canine *P. aeruginosa* isolates were cephalosporin resistant in 67% (8/12) of cases. Resistance to fusidic acid was observed in 17% (dogs 5/30), 7% (cats 1/12), and 50% (horses 2/4) of all *Staphylococcus spp*.

Only four isolates (three canine beta-hemolytic streptococci and one equine *S. pseudintermedius)* were resistant to all three agents in the triple-antibiotic combination of

Table 1. Bacterial isolates from canine, feline and equine eyes with septic keratitis

Isolate genus	Isolate species	Total isolates—canine eyes 113 (100%)	Total Isolates—feline eyes 32 (100%)	Total isolates—equine eyes 35 (100%)
Staphylococcus		46 (40.7%)	21 (65.6%)	12 (34.28%)
	S. pseudintermedius	11	3	1
	MRSP	3	1	1
	S. aureus	8	1	1
	Hemolytic *Staphylococcus* spp.	9	9	1
	Unspecified (*Staphylococcus* spp.)	15	7	8
Streptococcus		29 (25.66%)	4 (12.5%)	16 (45.71%)
	Beta-hemolytic *Streptococcus*	22	3	6
	S. canis	1	0	0
	S. equi ssp. zooepidemicus	0	0	3
	Unspecified (*Streptococcus* spp.)	6	1	7
Pseudomonas		12 (10.62%)	1 (3.1%)	2 (5.71%)
	P. aeruginosa	12	0	2
	Unspecified (*Pseudomonas* spp.)	0	1	0
Pasteurella		8 (7.08%)	2 (6.3%)	0
	P. multocida	7	1	0
	Unspecified (*Pasteurella* spp.)	1	1	0
Coliform + Escherichia		9 (7.96%)	1 (3.1%)	2 (5.71%)
Escherichia	*E. coli*	4	1	1
Coliform		5	0	1
Coryneform/Corynebacteria		3 (2.65%)	0	1 (2.85%)
Aerobic spore-formers		6 (5.31%)	2 (6.3%)	1 (2.85%)
	B. cereus	4	1	0
	Unspecified	2	1	1
Gram-positive anaerobic rods		0	1 (3.1%)	0
Enterococci		0	0	1 (2.85%)

An overview of the bacterial isolates collected from clinical patients examined by the ophthalmology section at the Vetsuisse Faculty, University of Zurich between January 2009 and December 2013.

neomycin, polymyxin B (colistin tested), and bacitracin. In several other cases, complete resistance could not be ruled out because one of the three antibiotics had either not been tested on the isolate (14 cases: six staphylococci, seven streptococci, one *P. aeruginosa*.; bacitracin not tested) or yielded an intermediate susceptibility result (seven cases).

Breed predispositions

Various breeds were represented in the study, among them eight brachycephalic dog breeds and one brachycephalic cat breed. The ORs for the presence of septic keratitis and binomial 95% confidence intervals in evaluated dog and cat breeds are listed in Table 2. Pugs had a particularly high chance to present with septic keratitis (OR: 70.39, CI: 42.49–116.62) compared to nonbrachycephalic breeds with a large representation in the general hospital reference population (Labrador Retriever: OR: 0.44, CI: 0.06–3.25; Mixed Breed: OR: 1.06, CI: 0.41–2.72). In a similar fashion, English Bulldogs, French Bulldogs, Pekingese, and Shih Tzus are at increased risk for having septic keratitis. Boston Terriers also had an increased OR for septic keratitis, although low case numbers resulted in a wide confidence interval. Persian cats also demonstrate a higher chance to be presented with septic keratitis (OR: 12.21, CI: 5.71–26.10) compared to Domestic Shorthair cats (OR 0.52, CI: 0.24–1.10), which is the most prevalent nonbrachycephalic breed in the reference population.

Six percent of all canine and 5% of all feline patients presented at the veterinary teaching hospital of the Vetsuisse Faculty, University of Zurich, were brachycephalic (OblonData Praxissoftware). In comparison, 59% (53/89) of the dogs and 39% (11/28) of the cats included in the current study were brachycephalic.

DISCUSSION

Staphylococci and streptococci accounted for more than 66% of bacterial isolates from dogs, cats, and horses with septic keratitis in this study. Almost 50% of the staphylococci isolated from dogs were resistant to second-generation fluoroquinolones. Brachycephalic animals were at particularly high risk of having septic keratitis.

Staphylococci and streptococci accounted for 66% of the bacterial isolates in dogs and 80% of the isolates in cats and horses The isolation rates of ocular surface bacteria and the percentages of negative cultures vary across studies. Our findings correspond with those from previous studies.[7,8,12,14–18,32–40] Some of the studies cited above-

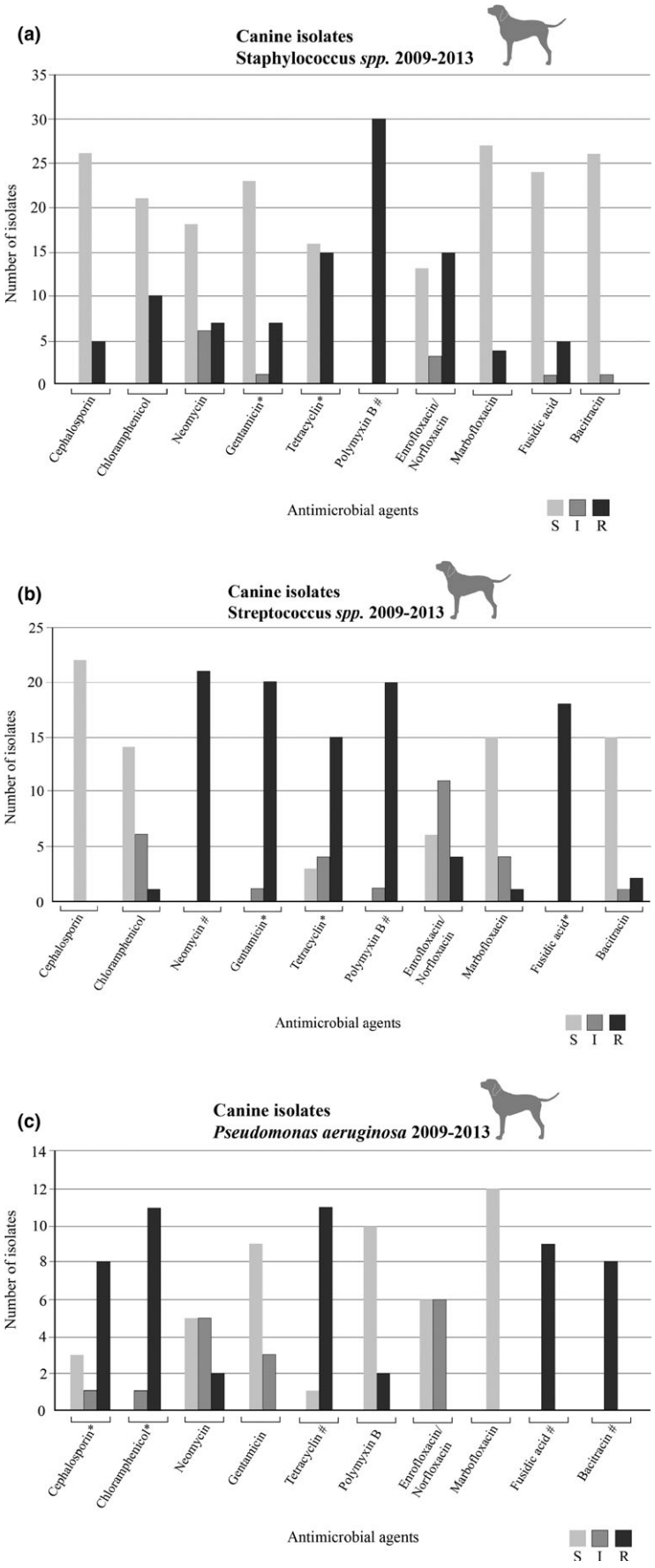


Figure 1. Antibiotic susceptibility of most commonly isolated bacterial pathogens from dogs with septic keratitis. (a) antibiotic susceptibility of *Staphylococcus spp.* isolated from dogs. (b) antibiotic susceptibility of *Streptococcus spp*. isolated from dogs. (c) antibiotic susceptibility of *Pseudomonas aeruginosa* isolated from dogs. Included were all *Staphylococcus spp.* known to be pathogenic; *S. pseudintermedius*, *S. epidermidis*, *S. aureus*, methicillin-resistant *Staphylococcus pseudintermedius*, and those with proven hemolysis (hemolytic staphylococci). Specified *Streptococcus* isolates from dogs were mostly beta-hemolytic *Streptococcus spp*., except one *S. canis* isolate. The total number of isolates is listed on the left. Not all antibiotics were tested on all isolates; therefore, the numbers in the bars differ from the total number of isolates. Antibiotics for which a variable effectivity against certain bacterial groups has classically been observed were marked '*: variable susceptibility' in the figures. Antibiotics against which, due to pharmacological mechanisms, resistance occurs naturally within certain bacterial groups were marked '#: intrinsic resistance' in the figures. Agar disk diffusion tests were interpreted in accordance with CLSI (clinical laboratory standards institute) standards or manufacturer-supplied breakpoints to define resistance as 'S': sensitive; 'I': intermediate sensitivity or 'R': resistant. Such breakpoints are based on concentrations that may be reached in plasma, and breakpoints for topically applied antibiotics have not been established. This is important because clinical susceptibility depends on the corneal concentration and bioavailability of topically applied antibiotics, which can lie orders of magnitude above safely reachable plasma concentrations.

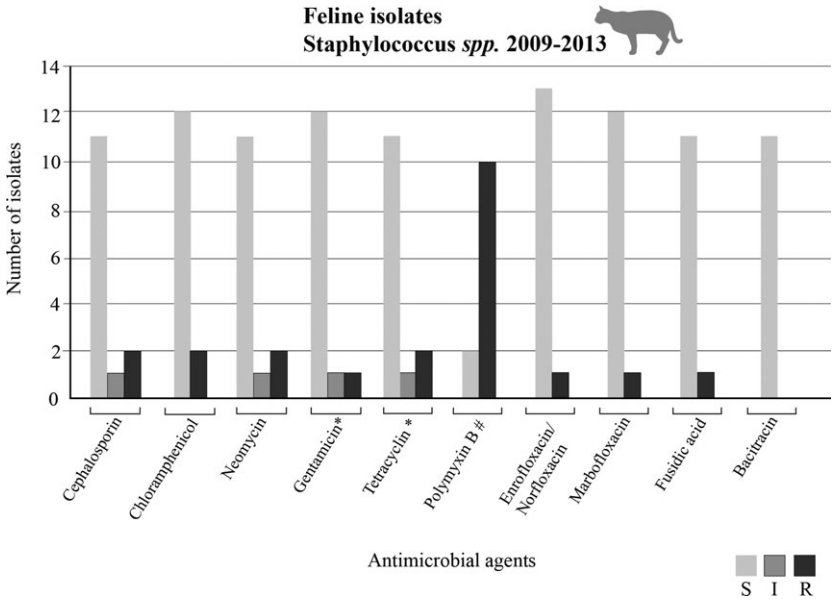


Figure 2. Antibiotic susceptibility of *Staphylococcus spp*. isolated from cats with septic keratitis. Fourteen pathogenic Staphylococcus isolates were analyzed: three *S. pseudintermedius*, one *S. aureus*, one methicillin-resistant *Staphylococcus aureus*, and nine other hemolytic *Staphylococcus spp*. Only three *Streptococcus* isolates were sufficiently specified to be included in the data analysis. Therefore, a graph was not assembled for these cases. The total number of isolates is listed on the left. Not all antibiotics were tested on all isolates; therefore, the numbers in the bars differ from the total number of isolates. Antibiotics for which a variable effectivity against certain bacterial groups has classically been observed were marked '*: variable susceptibility' in the figures. Antibiotics against which, due to pharmacological mechanisms, resistance occurs naturally within certain bacterial groups were marked '#: intrinsic resistance' in the figures. Agar disk diffusion tests were interpreted in accordance with CLSI (clinical laboratory standards institute) standards or manufacturer-supplied breakpoints to define resistance as 'S': sensitive; 'I': intermediate sensitivity or 'R': resistant. Such breakpoints are based on concentrations that may be reached in plasma, and breakpoints for topically applied antibiotics have not been established. This is important because clinical susceptibility depends on the corneal concentration and bioavailability of topically applied antibiotics, which can lie orders of magnitude above safely reachable plasma concentrations.

reported higher isolation rates for *P. aeruginosa* and lower isolation rates for beta-hemolytic streptococci in dogs[8,14] and higher isolation rates for *P. aeruginosa* in dogs [12] and in horses[15,16] in comparison with the current study. The differences in bacterial isolation rates reported in comparable studies[12,32,33] might be related to smaller study sample sizes, geographical/climatic differences, and/or differences in analytic and interpretational methods.

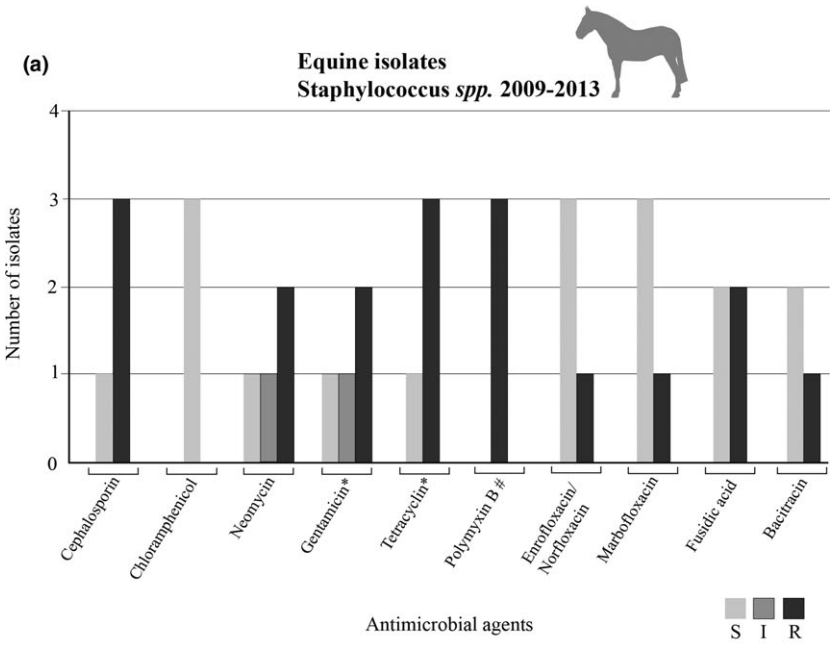
Almost 50% (15/31) of canine Staphylococcus isolates were resistant to the second-generation fluoroquinolones enrofloxacin and norfloxacin, which suggests an increase compared to previous reports. In 2006, Tolar *et al.*[8] found 100% of staphylococci and streptococci to be susceptible to enrofloxacin. Various reports in human medicine[20–23] clearly suggest increased fluoroquinolone resistance. However, results vary between studies and other groups report stable sensitivity to fluoroquinolones over time.[41–43] Bacterial resistance against third- and fourth- generation fluoroquinolones is still relatively rare but was observed in this study and can logically be expected to increase as has occurred for the first- and second- generation fluoroquinolones.[20,22,44]

Gentamicin is typically used against gram-negative pathogens, with special efficacy against *P. aeruginosa*,[19,45] which is supported by the results of this study. The reported susceptibility of gram-positive bacteria for gentamicin is variable.[7,8,15–17,19,45,46] Gentamicin resistance was observed in 95–100% of tested *Streptococcus* isolates in our study.

Cephalosporins have typically been effective against *Streptococcus* and *Staphylococcus spp.*[19,47] Whereas cephalosporin resistance was not observed in *Streptococcus spp.*, we did demonstrate cephalosporin resistance in 16% of the tested Staphylococcus isolates from dogs, which could be an indication of emerging cephalosporin resistance in *Staphylococcus spp.*

The same concern applies to the traditionally antistaphylococcal antibiotic fusidic acid,[19] to which 17% of all tested staphylococci in this study was resistant.

The intrinsic resistance of *Staphylococcus* and *Streptococcus spp.* to polymyxin B and of *Streptococcus spp.* to neomycin was confirmed with 100% of resistant isolates in this study. Effectivity against Staphylococcus isolates was variable for neomycin. Resistance to neomycin and polymyxin B was observed in 16% of canine *P. aeruginosa* isolates. These results support the reported variable neomycin resistance in *Staphylococcus spp.* and *Pseudomonas spp.* strains.[7,8] Pseudomonas isolates are routinely susceptible to polymyxin B, and acquired resistance reportedly is rare.[48]



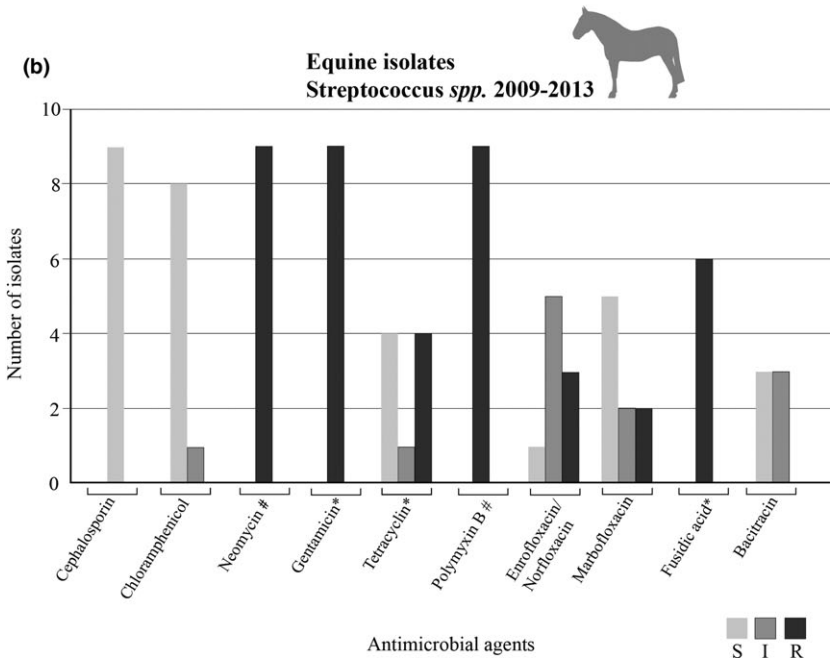


Figure 3. Antibiotic susceptibility of most commonly isolated bacterial pathogens from horses with septic keratitis. (a) antibiotic susceptibility of *Staphylococcus spp*. isolated from horses. (b) antibiotic susceptibility of *Streptococcus spp*. isolated from horses. The 4 equine Staphylococcus isolates in (a) included one *S. pseudintermedius*, one *S. aureus*, one methicillin-resistant *Staphylococcus pseudintermedius*, and one additional hemolytic Staphylococcus. The *Streptococcus* isolates in (b) included six beta-hemolytic *Streptococcus spp*. and three *S. equi ssp. zooepidemicus*. The total number of isolates is listed on the left. Not all antibiotics were tested on all isolates; therefore, the numbers in the bars differ from the total number of isolates. Antibiotics for which a variable effectivity against certain bacterial groups has classically been observed were marked '*: variable susceptibility' in the figures. Antibiotics against which, due to pharmacological mechanisms, resistance occurs naturally within certain bacterial groups were marked '#: intrinsic resistance' in the figures. Agar disk diffusion tests were interpreted in accordance with CLSI (clinical laboratory standards institute) standards or manufacturer-supplied breakpoints to define resistance as 'S': sensitive; 'I': intermediate sensitivity or 'R': resistant. Such breakpoints are based on concentrations that may be reached in plasma, and breakpoints for topically applied antibiotics have not been established. This is important because clinical susceptibility depends on the corneal concentration and bioavailability of topically applied antibiotics, which can lie orders of magnitude above safely reachable plasma concentrations.

Bacitracin is effective against gram-positive bacteria but ineffective against gram-negative organisms. *P. aeruginosa* has intrinsic resistance against bacitracin.[19] Interestingly, very few of our 180 bacterial isolates were proven to be resistant against the triple-antibiotic combination of neomycin, polymyxin B (colistin), and bacitracin (gramicidin). However, due to its toxic and anaphylactic potential, which precludes its systemic use in veterinary medicine, bacitracin was not included in the panel of tested antibiotics in 14 cases in this study. Most of the *Staphylococcus* and *Streptococcus spp*., which were isolated in 13 of these 14 cases, may have been sensitive to bacitracin as bacitracin resistance was rare among our gram-positive isolates.

In the present study, the odds ratios for the presence of septic keratitis were significantly increased for brachycephalic individuals, and especially for Pugs, compared to the general mesocephalic canine hospital population. For comparative and descriptive reasons, ORs were listed for all dog breeds represented in the study population. However, some of the ORs listed were likely coincidental, especially for breeds with only one individual representing the breed in the study population and low case numbers in the

Table 2. Odds ratios for the presence of septic keratitis (see main text for further details)

Breed	Cases	Reference hospital population	OR (confidence interval)	Breed-specific prevalence (Binomial 95% (confidence interval)
Alaskan Malamute	1	150	11.52 (1.56–84.59)	0.6% (0.01–3.6%)
Appenzeller	2	553	6.25 (1.50–26.02)	0.36% (0.04–1.2%)
Australian Shepherd	1	412	4.19 (0.57–30.67)	0.24% (0.006–1%)
Beagle	1	740	2.33 (0.31–17.06)	0.13% (0.003–0.7%)
Bloodhound	1	52	33.24 (4.47–246.99)	1.8% (0.04–10%)
Boston Terrier	3	85	61.01 (18.43–201.92)	3.4% (0.7–9.6%)
Boxer	2	1650	2.09 (0.50–8.70)	0.12% (0.01–0.4%)
Berner Sennenhund	1	1815	0.95 (0.13–6.95)	0.05% (0.001–0.3%)
Cairn Terrier	2	469	7.37 (1.76–30.70)	0.42% (0.05–1.5%)
Cavalier King Charles Spaniel	1	187	9.24 (1.26–67.77)	0.5% (0.01–2.9%)
Chihuahua	4	1074	6.43 (2.28–18.11)	0.3% (0.1–0.9%)
Cocker Spaniel	3	1209	4.28 (1.31–13.94)	0.2% (0.05–0.7%)
Continental Bulldog	1	139	12.43 (1.69–91.33)	0.7% (0.01–3.9%)
English Bulldog	4	273	25.32 (8.95–71.64)	1.4% (0.3–3.6%)
Flat Coated Retriever	1	1133	1.52 (0.20–11.13)	0.08% (0.002–0.4%)
French Bulldog	6	607	17.08 (7.17–40.7)	0.9% (0.3–2.1%)
Kleinspitz	1	27	64.02 (8.47–483.83)	3.5% (0.09–18.3%)
Labrador Retriever	1	3878	0.44 (0.06–3.25)	0.02% (0.0006–0.1%)
Luzerner Laufhund	1	41	42.16 (5.64–314.81)	0.2% (0.06–12.5%)
Maltese	1	404	4.27 (0.58–31.28)	0.2% (0.006–1.3%)
Mixed Breed	5	8086	1.06 (0.41–2.72)	0.06% (0.02–0.1%)
Pekingese	5	393	21.99 (8.58–56.33)	1.2% (0.4–2.9%)
Poodle	2	448	7.71 (1.85–32.14)	0.4% (0.05–1.5%)
Pug	27	663	70.39 (42.49–116.62)	3.9% (2.5–5.6%)
Shih Tzu	5	275	31.42 (12.24–80.69)	1.7% (0.5–4.1%)
Toy Terrier	1	13	132.97 (16.94–1043.32)	7.1% (0.1–33.8%)
Weimaraner	1	116	14.90 (2.02–109.59)	0.8% (0.02–4.6%)
West Highland White Terrier	2	1221	2.83 (0.68–11.77)	0.1% (0.01–0.5%)
Yorkshire Terrier	3	1571	3.30 (1.01–10.73)	0.1% (0.03–0.5%)
Persian Cat	11	1916	12.21 (5.71–26.10)	0.5% (0.2–1%)
Domestic Shorthair	16	25934	0.52 (0.24–1.10)	0.06% (0.03–0.1%)
Siamese	1	862	1.51 (0.20–11.14)	0.1% (0.002–0.6%)

Odds ratios for and breed-specific prevalence of septic keratitis in clinical patients examined by the ophthalmology section at the Vetsuisse Faculty, University of Zurich between January 2009 and December 2013.

reference population. In this study, the Toy Terrier breed, with one individual representing the breed in the study population and 13 dogs representing the breed in the reference population (OR: 132.97, CI: 16.94–1043.32), is an example of a likely coincidentally increased OR with a wide CI suggesting great statistical uncertainty. Packer *et al.*[49] reported that brachycephalism (20x), the presence of nasal folds (5x), a 10% increase in relative eyelid aperture width (3x), and exposed scleral tissue (3x) predispose dogs to the development of corneal ulceration by the multiplication factors listed between parentheses. Thus, factors contributing to a link between brachycephalism and septic keratitis likely include the combination of relative exophthalmos and macroblepharon that leads to lagophthalmos.

Limitations of this study include the following: CSLI or manufacturer-supplied breakpoints used to define antibiotic resistance are based on concentrations that may be reached in plasma, and breakpoints for topically applied antibiotics have not been established. This is important because clinical susceptibility depends on the corneal concentration and bioavailability of topically applied antibiotics, which can lie orders of magnitude above safely reachable plasma concentrations.[25] Therefore, bacterial isolates that are determined to be 'intermediately susceptible' or 'resistant' based on agar disk diffusion susceptibility testing with CLSI breakpoints can be clinically susceptible to the antibiotic in question.

Various groups have presented minimal inhibitory concentrations (MICs) for combinations of bacterial isolates and antibiotics.[24,50,51] Kaye *et al.*[24] have demonstrated an association between the MICs for certain bacteria/antibiotic combinations and the clinical outcome for human patients with septic keratitis. The availability and presentation of minimal inhibitory concentrations (MICs) for the isolate/drug combinations tested in this study would therefore have been preferable. However, MIC values were not available during the timeframe of the study as the test results were reported by the diagnostic laboratory as S (sensitive), I (intermediate), or R (resistant) without actual inhibition zone measurements.

Despite the fact that the agar disk diffusion susceptibility test is a scientifically proven process which yields accurate results with well-trained laboratory personnel, a certain interobserver test bias as a result of an inherent variability and potential subjectivity of manual inhibition zone measurement needs to be taken into account. An automated readout process would have eliminated such potential interobserver variability of measurements within the study and would potentially have increased the comparability to other literature sources. The high number of isolates classified as intermediate in some bacterial species for certain antibiotic combinations might thus be explained.

Due to the referral nature of the University of Zurich's veterinary ophthalmology clinic, almost all of the patients were premedicated in some way, which might contribute to higher resistance rates and a higher number of negative cultures than in the actual general patient population.

Finally, only isolates that were clearly labeled on species level and with known pathogenicity and those with proven hemolysis were included in the evaluation of the antibiotic susceptibility test results. This resulted in a decreased study sample size for this part of the study.

In summary, the elevated odds ratios for septic keratitis in brachycephalic breeds and the bacterial isolates identified in this study are consistent with previous studies. The observed pathogen resistance against antimicrobial agents (e.g., second-generation fluoroquinolones, cephalosporins, potentially fusidic acid) is concerning and might be reduced by prudent and, where possible, targeted use of antibiotics. The use of neomycin–polymyxin B-bacitracin/gramicidin triple-antibiotic combinations might be a valid alternative to the first-line use of fluoroquinolones or aminoglycosides for the treatment of septic keratitis.

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